Interaction between filaggrin mutations and neonatal cat exposure in atopic dermatitis

Letter to the editor

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Introduction

Atopic dermatitis (AD) is a prevalent inflammatory skin disease. Loss-of-function mutations in filaggrin gene (*FLG*) represent the strongest genetic risk factors for AD, being strongly associated with early disease onset and persistence into adulthood.[1] The epidermis of individuals with mutations in *FLG* is fundamentally different from normal skin being characterized by increased penetration of allergens.[2]

Recent birth cohort studies showed a significant interaction between cat ownership at birth and mutations in *FLG* (R501X, 2282del4) on the development of early-onset AD.[3] This finding was replicated for the 2282del4 *FLG* mutation in a Dutch cohort study, and extended to further associate with risk of allergic sensitization.[4] We performed analyses in multiple birth cohorts to examine the consistency and overall strength of the previously observed interaction.

Materials and methods

Cohorts, exposures, genotypes and phenotypes

Consortium collaborators were invited to participate in the study, [5] and 13 birth cohorts provided data on cat exposure, AD, and filaggrin mutations (Table 1 and Supplementary Table 1+2). All cohorts had information on the most common mutations in *FLG*, R501X and 2282del4, and the majority also had information on R2447X and S3247X (Table 1). Heterozygous, compound heterozygous and homozygous *FLG* mutation carriers were pooled as mutation carriers. Cat ownership/exposure was based on questionnaires or interviews. AD diagnoses were based on questionnaires in 11 cohorts, and by physician examination in 3 cohorts (COPSAC2000, COPSAC2010 and MAAS). For further details, please see supplementary information online.

Statistical analysis and outcomes

The predetermined primary outcome was AD onset before one year of age ('AD_{early}').Secondary outcomes included i) current AD at seven years of age or the year of assessment closest to, but before, 7 years ('$AD_{current}$'), and ii) a history of AD during the first 7 years of life, or last year of assessment (AD_{ever}). For further details, please see supplementary information online.

Results

A total of 22,133 children were studied (Table 1 and supplementary Table 1). The median prevalence (range) of mutations in *FLG* was 9.4% (4.6-12.3), cat exposure 15% (7.9-29.6), $AD_{early)}$, $AD_{current}$, and AD_{ever}, respectively, 18% (9.7-34.6), 13.9% (3.9-20), and 39.5% (20.4-67). There was no interaction between *FLG* mutations and cat exposure on the risk of the primary outcome 'AD_{early}' (OR 1.10 (95% CI 0.86-1.43), I^2% 0.0), (Figure 1 and Table 1). There was a statistically significant interaction for the secondary outcome of having AD at last time of examination or questioning at 7 years of age ($AD_{current}$), in the direction of increased risk of AD from cat exposure in children with *FLG* mutations (OR 1.36 (95% CI 1.02-1.82) I^2% 8.6), but this was not statistically significant after adjustment for multiple testing. The *FLG*-stratified analyses showed a trend towards cat exposure being a risk factor in children with *FLG* mutations and a protective factor in children without *FLG* mutations (Figure 1 and Supplementary Table 3). No interaction was found for the other secondary outcome 'AD_{ever}' (OR 1.06 (95% CI 0.82-1.37), I^2% 0.0)

Discussion

We found no interaction between cat exposure in infancy and mutations in *FLG* on 'early-onset AD' or 'AD ever'. A nominally significant interaction in the expected direction was found for the secondary outcome 'current AD' at 7 years of age, but this did not survive adjustment for multiple testing.

A particular study strength is the large number of independent birth cohorts with prospective assessment of exposure and outcomes. Most cohorts had genotype information for the 4 most common *FLG* mutations ensuring a high degree of correct classification. AD diagnoses were based on questionnaire data in most cohorts, potentially reducing diagnostic specificity. Since AD is a chronic and relapsing disease, short episodes of other eczemas, e.g. due to irritant or allergic contact dermatitis, may be suspected of being AD by parents and caregivers, in particular in the first years of life where flexural accentuation is not yet occurring. One may argue that AD measured at 7 years of age is expected to have a higher specificity due to flexural involvement. [6]. Notably, the high prevalence of early AD in some cohorts could mask a true cat exposure-*FLG* mutation

interaction. Cat exposure was only assessed around birth, and it is possible that later exposure to cat could have an unmeasured effect on AD. Similar, the extent of cat exposure might vary between studies and families. Other environmental factors were not included since covariate availability differed between the cohorts. Reverse causality cannot be excluded, as families who had experienced atopic disease might have avoided having pets to prevent allergic disease in their (next) child. However, one would expect families with *FLG* mutations, and thereby increased risk of eczema, to avoid cat ownership, which would tend towards an apparent protective effect of having a cat.

No association between cat ownership and AD was found in another meta-analysis of 13 studies (relative risk 0.94 (95%CI 0.76-1.16)).[7] When a compelling gene-environment interaction was observed between *FLG* mutations and cat ownership on the risk of early-onset AD in birth cohorts, it raised the possibility that preventive measures against pediatric AD could be identified by taking the genetic susceptibility into account.[3, 4] The COPSAC2000 study, which provided the basis for the previous report of interaction between cat and *FLG* mutations, [3] benefited from close follow-up of children, and high AD diagnostic accuracy, whereas most birth cohorts in the present meta-analysis used questionnaires, potentially leading to misclassification.

No pathomechanism has been established for the proposed association between cat ownership and AD in *FLG* mutation carriers. Possibly, very small cat allergens might penetrate into the viable layers of the epidermis, where they can exert immune effects.[8] Previous studies demonstrating increased risk of peanut allergy *FLG* mutation carriers, also suggest increased peanut allergen skin penetration. [9] Another explanation could be an effect of cat exposure on the gut microbiome of mothers and children. However, the absence of filaggrin protein expression in the gut argues against this.

In conclusion, this meta-analysis could not confirm an interaction between cat exposure in infancy and *FLG* mutations on development of early-onset AD. Gene-environment interactions remain largely unknown.

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Table 1. Baseline characteristics of participants in birth cohorts.

Cohort	ALSPAC	BAMSE	Baseline	COPSAC 2000	COPSAC 2010	DNBC	Generation R	GINIplus	INMA*	LISA	MAS	MAAS	RAINE
Cohort inclusion criteria	Children from the general population	Children from the general population	Children from the general population	Children born from mothers with asthma	Children from the general population	Children from the general population	Children from the general population	Children from the general population	Children from the general population	Children from the general population	Children from the general population	Children from the general population	Children from the general population
Study year baseline	1991-1992	1994-1996	2008	2000	2010	1996-2001	2002-2006	1995-98	1997-2006**	1997-99	1990	1996-1997	1989-1991
Filaggrin mutations genotyped	R501X, 2282del4, R2447X, S3247X	R501X, 2282del4, R2447X	R501X, 2282del4, R2447X, S3247X	R501X, 2282del4, R2447X, S3247X	R501X, 2282del4, R2447X, S3247X	R501X, 2282del4, R2447X, S3247X	R501X, 2282del4, R2447X, S3247X	R501X, 2282del4	R501X, 2282del4, R2447X, S3247X	R501X, 2282del4	R501X, 2282del4, R2447X, S3247X	R501X; 2282delL4, R3247X, R2447X	R501X, 2282del4, S3247X, rs138726443
Proportion with *FLG* mutations	11% (834/7743)	7.2% (138/1906)	10.8% (146/1344)	12.3% (49/396)	10.3% (72/700)	9.7% (91/935)	9.4% (268/2849)	7.0% (104/1490)	4.6% (28/606)	7.0% (69/987)	9.7% (79/813)	10.1% (87/864	9.3% (140/1500)
Basis for atopic dermatitis diagnosis	Questionnaire	Questionnaire	Questionnaire and clinical diagnosis	Clinical diagnosis	Clinical diagnosis	Questionnaire	Questionnaire	Questionnaire	Questionnaire	Questionnaire	Questionnaire and clinical diagnosis	Questionnaire	Questionnaire
AD 'early onset (≤1y)'	18% (1368/7743)	16.9% (323/1906)	22.7% (292/1282)	25.3% (100/396)	11.1% (78/700)	14.7% (137/935)	21.6% (545/2521)	11.3% (167/1477)	31.7% (192/606)	9.7% (94/973)	14.14% (115/813)	34.6% (160/462)	22.8% (341/1498)
AD 'current'	20% (1270/6402)	17.2% (327/1896)	15.2% (178/1168)	13.9% (55/396)	13.6% (95/700)	6.8% (65/963)	18.6% (496/2658)	5.93% (77/1298)	19.5% (118/604)	3.9% (32/825)	7.5% (58/773)	14.1% (96/681)	13.3% (184/1386)
AD 'ever (0-7 y)'	67% (4367/6501)	39.5% (743/1878)	26.3% (354/1344)	42.2% (175/396)	27.6% (193/700)	20.4% (196/963)	41.4% (1180/2849)	35.3% (447/1266)	49.6% (307/618)	32.6% (269/826)	36.2% (294/813)	60.7% (306/504)	39.7% (596/1500)
AD assessment time-points	6, 18, 30, 42, 57, 69, 81 months	1, 2, 4 and 8 years	6, 12 and 24 months	1 month, and then every 6 months.	1 month, and then every 6 months.	6 and 18 month, and 7 years	6 months, and 1, 2, 3, 4 and 6 years	1, 2, 3, 4, 6 and 10 years	1, 2, and 4 years	6, 12, 18, 24 months and 4, 6 and 10 years	1, 3, 6, 12, 18, 24 month and then yearly	1,3,5,8 years	1, 3, 5, 8 years
Child age at cat exposure assessment	During pregnancy	At baseline (3 months) and/or 1 year follow-up.	6 months and 12 months	Birth	Birth	18 months	age < 1 year)	1 year	1 year	3 months and 1 year	3 months	Birth	1 year
Early life cat exposure %	29.6	10.6**	8.8	15	20	16.4-22.4 **	25.3	7.9	11.5	12.2	12.7	20.5	18

*INMA sub cohorts: VAL, SAB, MEN

** please see supplementary Table 1 for further details.

Figure 1. Interaction between cat exposure and common *FLG* mutations in relation to a) Early onset atopic dermatitis, b) Current atopic dermatitis and c) atopic dermatitis in the first 7 years of life.